Unintentionally Released *Chaetorellia succinea* (Diptera: Tephritidae): Is This Natural Enemy of Yellow Starthistle a Threat to Safflower Growers?

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ABSTRACT In mid-1996, we detected an unintentionally introduced seed-head fly, *Chaetorellia succinea* (Costa), destroying seeds of yellow starthistle, *Centaurea solstitialis* L., one of the worst weeds in the western United States. In overseas studies, *Chaetorellia succinea* had been considered as a potential biological control agent for yellow starthistle, but had been rejected because of fears that it might become a pest of safflower, *Carthamus tinctorius* L., in the United States. From mid-1996 through early 2000, we conducted both laboratory and field evaluations to determine whether this fly could cause significant damage to safflower, a widely planted crop in California. In laboratory no-choice host range evaluations, adult females would oviposit, and the larvae completed development, on all five varieties of safflower that we tested. However, in choice tests, only one head each of two varieties of safflower was attacked. No safflower was attacked at three sites in California and Oregon, with large populations of *Chaetorellia succinea,* where we grew five varieties of safflower as 'trap plants.' Our monitoring of possible *Chaetorellia succinea* attack on safflower growing in 47 fields in California detected a small, but persistent population of this fly infesting an uncommon safflower variety at one field. We feel that our results indicate a minimal risk to commercial safflower growers, and this fly continues to show promise in assisting toward the eventual biological control of yellow starthistle.

KEY WORDS *Centaurea solstitialis*, *Carthamus tinctorius*, biological control of weeds, realized host range, choice tests, nontarget impact

YELLOW STARTHISTLE, *Centaurea solstitialis* L., is an exotic weed that is native to the eastern Mediterranean region. The prickly spines that surround its inflorescences interfere with grazing by cattle, thereby greatly diminishing forage values and economic returns from rangelands and pastures. Horses (but not other livestock) that graze on yellow starthistle can develop a fatal neurological disorder called nigropallidal encephalomalacia (Cordy 1978) or "chewing disease" (Fuller and McClintock 1986). Yellow starthistle also reduces biodiversity by displacing native plants in grasslands, wildlands, orchards and vineyards, roadsides, and waste places. In North America, it is now established in 23 of the 48 contiguous states, and in Canada, from British Columbia to Ontario (USDA-ARS 1970). It is most widespread and pernicious in California and the Pacific Northwest. During a 1997 survey, Pitcairn et al. (1998b) found it present in 56 of California's 58 counties, and in 1,935 of its 4,638 townships. Yellow starthistle has also invaded the southern hemisphere, primarily in areas with Mediterranean climates, such as Australia (Lamp and Collet 1979), New Zealand (Webb et al. 1988), and South Africa (Wells et al. 1986).

By the late 1950s, the U.S. Department of Agriculture had added yellow starthistle to the list of weeds that their overseas scientists targeted in surveys for potential classical biocontrol candidates (Balciunas 1998). These overseas surveys and research, coupled with the final screening of candidate insects at the USDA-ARS quarantine laboratory in Albany, CA, eventually led to the release of six insects, all of which destroy or inhibit developing seeds in the inflorescences of yellow starthistle. The first, a gall forming fly, *Urophora jaculata* Rondani (Diptera: Tephritidae) from Sicily, was released in 1969, but failed to establish (Maddox 1981, Ehler and Andres 1983). The next five insects–two flies (Diptera: Tephritidae): *Urophora sirunaseva* (Hering) and *Chaetorellia australis* Hering; and three weevils (Coleoptera: Curculionidae): *Bangasternus orientalis* (Capiomont), *Eustenopus villosus* (Boheman), and *Larinus curtus* Hochhut–all from Greece, established in several states (Turner et al. 1995, Pitcairn et al. 1998a).

During our 1995 and 1996 surveys to record the establishment and distribution of *Chaetorellia australis*, we detected the presence of another, very similar

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fly (Balciunas and Villegas 1999). *Chaetorellia australis,* first released in 1988, appeared to be establishing only at sites where an alternate host, bachelor button (*Centaurea cyanus* L.), was also present, and it was theorized that bachelor button was required as an early season host for *Chaetorellia australis* (Turner et al. 1995). However, during our 1995 and 1996 surveys, we recovered *Chaetorellia* flies at many California sites where bachelor button was absent (Balciunas and Villegas 1999). This suggested the possibility of a taxonomic problem with the *Chaetorellia* flies. Two tephritid taxonomists in California, after viewing our specimens, confirmed that most were not *Chaetorellia australis* (Balciunas and Villegas 1999).

We sent a comprehensive series of *Chaetorellia* flies to Ian White at the British Museum of Natural history, the coauthor of the most recent revision of the genus *Chaetorellia* (White and Marquardt 1989). After examining our specimens, Dr. White identified the second species as *Chaetorellia succinea* (Costa). After reviewing voucher specimens at the quarantine facility in Albany, CA, and those held by cooperators who had assisted in the releases of *Chaetorellia australis,* we determined that the most probable source of the USA introduction of *Chaetorellia succinea* was a 1991 shipment of *Chaetorellia australis* infested yellow starthistle heads from Greece (for a more complete discussion of the detection and unintentional release of *Chaetorellia succinea,* see Balciunas and Villegas 1999). This shipment contained a mixture of *Chaetorellia* species that went undetected before their release in Merlin, OR. Both species established there, and this Merlin site was subsequently used by many agencies from several western states as a source for redistribution of *Chaetorellia australis.* Furthermore, we found that *Chaetorellia succinea* flies are excellent dispersers, and documented their rapid spread throughout most of California (Balciunas and Villegas 1999).

Chaetorellia spp. females oviposit on the developing inflorescences (hereafter referred to as 'heads') of plants in the tribe Cardueae. The eggs of both *Chaetorellia australis* and *Chaetorellia succinea* are deposited on the outside of the developing heads, and the emerging neonates burrow into the heads, then the larvae feed within a single head on receptacle tissue and on the developing seeds (White and Marquardt 1989; J.K.B., unpublished data). After detecting this 'new' *Chaetorellia* in mid-1996, we immediately curtailed further releases of *Chaetorellia* species, and began investigating the safety of the unintentionally introduced *Chaetorellia succinea.* Several decades earlier, overseas scientists (Zwölfer 1972, Sobhian and Zwölfer 1985) had conducted preliminary host range tests of *Chaetorellia succinea,* at that time referring to it as *Chaetorellia* species near *carthami. Chaetorellia carthami* Stackelberg is a pest of safflower (*Carthamus tinctorius* L.) in western Asia and northern Africa, and these researchers devoted most of their efforts to determine the acceptability of this economic plant as a potential host for *Chaetorellia succinea.* Although in their tests, *Chaetorellia succinea* greatly preferred to oviposit on yellow starthistle, it could sometimes be induced to oviposit and develop on safflower. It would also mate and hybridize with *Chaetorellia carthami.* They, therefore, recommended against further consideration of *Chaetorellia succinea* for importation to the United States as a potential biological control agent for yellow starthistle (Sobhian and Zwölfer 1985).

Safflower is an important crop in California. During 1999, in California, 50,464 hectares of safflower were harvested, and this crop was valued at $44,187,000 (CASS 2000). Most of this safflower is grown in the Central Valley, where yellow starthistle is abundant, and exposure to *Chaetorellia succinea* is inevitable. In this article, we describe the results of our studies as follows: (1) to determine susceptibility of various safflower varieties, under both field and laboratory conditions, to attack by this fly, (2) to determine, by monitoring safflower fields in California, the impact, if any, this fly is having on safflower cultivation, (3) to compare the seed reduction attributed to *Chaetorellia succinea* infesting safflower with the damage this same fly causes to yellow starthistle, and finally (4) to compare the incidence of *Chaetorellia succinea* emerging from field-collected safflower with that of other herbivores emerging from the same heads.

Materials and Methods

Due to the importance of safflower, and the potential for damage by *Chaetorellia succinea,* we carried out laboratory and field evaluations to assess the susceptibility of different safflower varieties to attack by *Chaetorellia succinea.* These included four of the current most commonly grown commercial varieties in California (SeedTec variety 317, and three CalWest varieties: 88-ol, 1221, and 4440), a formerly popular commercial variety (SeedTec 541), and three noncommercial varieties (Cargill 44, 'Golden Orange', and 'Birdseed'). Commercial varieties are harvested for their seed from which oil is extracted. Noncommercial varieties are usually not harvested, but are grown as food for birds and wildlife, or to "dry out" moist soil.

We distinguished *Chaetorellia succinea* from other *Chaetorellia* spp. by the presence of an extra spot on each side of the thorax. White and Marquardt (1989) place the nine known species of *Chaetorellia* into two groups, with *Chaetorellia succinea* belonging to the *Chaetorellia loricata* group, and *Chaetorellia australis* to the *Chaetorellia jaceae* group. *Chaetorellia succinea* adults (and the other two species of the *Chaetorellia loricata* group) have one (sometimes two) additional "spots" on posterior dorsolateral portion of each side of their thoraxes, leading to an aggregate of 10 (sometimes 12) spots on the entire thorax. These additional spots are lacking in *Chaetorellia australis* along with the other five species in its group, which have only eight spots on their thoraxes. Because no other members of the *Chaetorellia loricata* group have been recorded in North America, we use these extra "spots" as an easy way to distinguish *Chaetorellia succinea* from all other *Chaetorellia* flies found here (for color photo illustrating this difference, see Balciunas and Villegas 1999). We have deposited voucher specimens of some of our *Chaetorellia succinea* in the United States National Museum at the Smithsonian in Washington, DC, and in the California State Collection of Arthropods in Sacramento, CA.

Laboratory Tests. Our laboratory evaluations of the acceptability of these different varieties of safflower were conducted at the USDA-ARS quarantine laboratory in Albany, CA. The laboratory tests began in mid-1996, and continued through early 2000. Unless otherwise noted, the flies used in our laboratory tests were newly-eclosed adults emerging from yellow starthistle or safflower heads collected at our study sites in California. *Chaetorellia* will oviposit only on flower heads at the appropriate stage (still closed, but approximately a week before anthesis). Following the widely used flower stage designations of Maddox (1981), the heads of both safflower and yellow starthistle used in our tests were BU-3s and BU-4s. The required overlap of the appropriate stage of safflower test plants, our yellow starthistle controls, and availability of newly emerged *Chaetorellia succinea,* was difficult to arrange, and occurred only a few times each year.

We used sleeve cages (73 by 42 by 49 cm) for most of the no-choice tests. The choice tests required larger cages, either a wooden-framed cube (100 by 100 by 100 cm) or metal screen cage (122 by 91 by 91 cm). In no-choice tests, the flies (n = 7–12 pairs) were exposed to one variety of safflower. Our first two no-choice experiments were conducted with a single, simultaneous no-choice yellow starthistle "control." For subsequent no-choice tests, the surviving flies from the no-choice test on safflower were subsequently exposed to a paired yellow starthistle "control" to verify that the surviving flies were indeed fertile. The choice tests were similar, except that the flies (n = 8–20 pairs) were simultaneously exposed to the yellow starthistle in the same cage with the safflower plant. Tests durations were generally two to three weeks to allow sufficient time for the younger heads to reach the appropriate stage, and for *Chaetorellia succinea* females to mature. The flies were then removed and the safflower and yellow starthistle controls kept in separate cages for at least three weeks to allow any *Chaetorellia succinea* larvae to complete development and emerge as adults. The safflower and yellow starthistle heads were then cut off the plants, held for several more weeks in emergence boxes to allow for late emerging flies. Emergence boxes were closed cardboard boxes (either 36 by 36 by 36 cm, or 32 by 24 by 36 cm) with a clear vial partially inserted into one side. After flies ceased to emerge in the boxes, we dissected all the heads of both safflower and the yellow starthistle controls. The contents of each head was examined, and we considered a head as 'infested' or 'damaged' if it had larval feeding damage, an empty pupal case, or a dead or overwintering larva. Multiple larvae in one head were counted as one infested head. Because, in our dissections of heads used in our laboratory tests, dead larvae were infrequently found (1–2%), we consider larval damage to indicate successful oviposition and development by *Chaetorellia succinea* on the host.

Because the number of female *Chaetorellia succinea* used in each test was not constant, we divided the number of damaged heads by the number of females used in each test. For each choice and no-choice test, we then compared the damaged heads per female of safflower against the paired yellow starthistle control using Pearson's chi-square test (SPSS 1997). To transform these values to an integer suitable for chi-square analysis, we multiplied the number of damaged heads per female $\times$ 100, then rounded to the nearest whole number. We also used this same procedure to analyze if *Chaetorellia succinea* reared from safflower damaged more heads than those reared from yellow starthistle.

To better illustrate if one of the safflower varieties was more susceptible to damage from *Chaetorellia succinea,* we pooled the infested heads per female data for each variety, then performed Pearson's chi-square test (SPSS 1997) comparing all pairs of varieties under both no-choice and choice conditions. We used the same analysis to compare each pooled variety against its pooled yellow starthistle control.

Field Evaluations. Our field evaluations were of two types–growing safflower as 'trap plants', and monitoring existing safflower fields. During spring and early summer of 1997, we planted an assortment of safflower varieties (usually 100 plants per variety), in small (several square meter) plots surrounded by yellow starthistle, at three sites in Josephine County, OR, and at a site near Yountville, in Napa County, CA. During 1996, we had confirmed that these four sites had both yellow starthistle and bachelor button present, and that they were heavily infested ($>$40%) by *Chaetorellia succinea* and *Chaetorellia australis,* respectively. At one of the Oregon sites, our safflower trap plants were heavily grazed by deer, and this site was dropped from the study. After the safflower at the remaining three sites had flowered and was beginning to dry up, it was harvested, the heads clipped off, and each variety/site kept in individual emergence boxes that were monitored for emerging insects. During the fall and winter, most of the safflower heads were dissected, to detect any overwintering flies, or those that had died or been parasitized.

Our second type of field study, involved monitoring safflower growing in 47 fields in 21 counties in California. At each field site, after the safflower had finished flowering, one of us would walk a transect through a field, and at every other step, collect the safflower plant closest to his shoe. The number of heads and the height of the plant was recorded, then the heads clipped off and saved in a bag. This was repeated until $\approx$300 heads had been collected in the bag. The heads were then transferred to emergence boxes. Because some *Chaetorellia succinea* larvae might overwinter, these boxes were monitored for emergence for 10–12 mo.

If a *Chaetorellia succinea* emerged from a safflower sample, we returned to the site within three weeks of the initial collection, and collected an additional, much larger sample of 2,000–3,000 heads. At Red Bluff, one of our safflower sites from which *Chaetorellia succinea* emerged, during 1998, we further stratified our large sample to determine if the ovipositing flies preferred safflower heads at a certain height, or of a particular size class. After cutting a safflower plant off at ground level, we measured its height, then cut it into four equal pieces. The top (fourth) quarter was further subdivided into the uppermost 10 cm layer (containing most of the heads), and top quarter remainder. Heads from each plant height were sorted into three size classes (small <1 cm diameter, medium 1–2 cm, large >2 cm), then placed in separate emergence cages. The data from this collection were analyzed to detect a preference for safflower bud height, and preference for bud size, using the Z-test for proportions (SPSS 1997) in both cases.

To determine the amount of damage caused by the larvae of *Chaetorellia succinea* to the safflower at our Red Bluff site, we dissected a portion of the safflower heads we had collected from there in 1997 ($n = 145$), 1998 ($n = 403$), and in 1999 ($n = 102$). We counted the seeds in damaged and undamaged heads, then compared the number seeds in infested versus undamaged heads, using Mann-Whitney Rank Sum Test (SPSS 1997). To put the safflower damage in perspective, we repeated this procedure on 262 yellow starthistle heads we collected in 1999, about hundred meters from the safflower at the Red Bluff site.

Because the Red Bluff site was the only safflower field at which we found significant number of flies during our 1997 and 1998 sampling, this was the only safflower field we monitored during 1999.

We found many other arthropod species attacking safflower heads at our 47 sites. We had the more commonly encountered species identified, and tabulated their relative abundance. For each of the 47 sites we assigned a value of 0 if the arthropod was absent, and one if the arthropod was present. We used Kruskal-Wallis one-way analysis of variance (ANOVA) on ranks (SPSS 1997) to confirm that there was a significant difference in frequency among the five arthropods, followed by Student-Newman-Keuls method (SPSS 1997) to determine which arthropod frequencies differed significantly.

Results

Laboratory Tests. In the eight no-choice tests (Table 1), *Chaetorellia succinea* oviposited and their larvae completed development on all five safflower varieties we tested. Except for two no-choice tests (CH-2–98 and CH-7–98), the number of heads infested per female on the yellow starthistle controls was always greater than on any safflower variety at a very highly significant level ($P < 0.001$). Interestingly, both of the "nonsignificant" tests used *Chaetorellia succinea* reared from field-collected safflower. We, therefore, then compared these two tests with tests (CH-2–96 and CH-12–99) on the same safflower varieties exposed to *Chaetorellia succinea* that had been reared from yellow starthistle. The safflower-reared flies infested more

Table 1. Damage by larvae infesting five safflower varieties and paired yellow starthistle controls exposed to *Chaetorellia succinea* adults under no-choice conditions

Test		*Ch. succinea*			Safflower			Yellow starthistle control			
Test no.	Test duration (days)	Population	n (♀)	Variety	Total heads	% of heads infested by *C. succinea*	Infested heads/♀	n (♀)	Total heads	% of heads infested by *C. succinea*	Infested heads/♀	χ^2
CH-1-96	63	RC	12	CalWest 4440	11	18	0.17	12[a]	274	41	9.40	18.03***
CH-2-96	63	RC	12	SeedTec 541	15	0	0.00					82.76***
CH-2-98	14	RB	12	Cargill 44	13	17	0.25	4	43	26	2.75	0.92
CH-7-98	24	RB	12[b]	SeedTec 541	15	13	0.17	4	29	17	1.25	0.77
CH-12-99	36	W	7	Cargill 44	5	20	0.14	5			6.57[c]	13.25***
CH-21-99	31	WC	7	CalWest 1221	16	25	0.57	6	58	67	6.50	134.37***
CH-27-99	21	WC	10	CalWest 88-o1	14	21	0.30	2	29	45	6.50	27.61***
CH-28-99	21	WC	10	CalWest 1221	15	7	0.10	0			6.57[d]	71.01***

Chaetorellia succinea populations: (all reared from yellow starthistle except RB, flies reared from safflower, and RC, flies swept from yellow starthistle) RC - Rancho Cordova, Sacramento County, CA. RB = Red Bluff, Tehama County, CA. W = Willow Creek, Humboldt County, CA. WC = Wildcat Canyon, Contra Costa County, CA. *** $P < 0.001$; chi-squared test of infested versus noninfested heads per female, df = 1.

[a] In the first two tests, one yellow starthistle control test using different flies, was run simultaneously with the two *Chaetorellia succinea* no-choice oviposition tests on safflower. For remaining tests, the yellow starthistle control was exposed to flies that had survived the no-choice oviposition test.

[b] Initially, 12 males and 12 females but all died or escaped; 7 d after the start of the experiment, 5 males and 12 females added.

[c] No flowering yellow starthistle available; for chi-square analysis used pooled yellow starthistle damage data from other no-choice tests.

[d] No female *Chaetorellia succinea* survived from the safflower portion of the test; used pooled yellow starthistle damage data from other no-choice tests.

safflower heads per female ($\chi^2 = 15.2$, df = 1, $P < 0.001$) of SeedTec 541 than did the flies reared from yellow starthistle, although there was no statistical difference for the other variety, Cargill 44 ($\chi^2 = 0.264$, df = 1, $P = 0.607$).

In the nine choice tests, when both yellow starthistle and a safflower variety were in the same cage, infested heads per female on yellow starthistle was always significantly greater (Table 2). In fact, only two safflower heads—one head each of CalWest 88-ol and Cargill 44—were infested out of the 208 that were exposed to *Chaetorellia succinea* during the nine choice tests.

The susceptibility of the five varieties of safflower, and of yellow starthistle, under both choice and no-choice conditions, is graphically illustrated in Fig. 1. For all five varieties, under both choice and no-choice conditions, the pooled infested heads per female was always very highly significantly lower ($P < 0.001$), when compared with its pooled yellow starthistle control. chi-square comparison of each variety's pooled damaged heads per female results from the no-choice tests, indicated that SeedTec 541 had significantly less damage ($P = 0.019$ to $P < 0.001$) when compared against each of the other four varieties.

Field Evaluations. In our 'trap plant' study at sites in Oregon and California, we grew five varieties of safflower in small plots surrounded by yellow starthistle that was heavily infested by *Chaetorellia succinea.* Although at some sites we lost many safflower heads to deer grazing, we were able to recover adequate heads from three sites. At our site near Yountville, CA, we harvested 90 and 238 safflower heads, respectively, of varieties CalWest 88-ol and CalWest 1,221, 97 heads of Bird Seed and 43 heads of Golden Orange. At our Merlin, OR site, we harvested 59 and 92 heads of the two CalWest varieties, and 371 heads of SeedTec 317; whereas at our Hampden Road site (also near Merlin), we recovered only 14 heads of the two CalWest varieties, and 56 heads of the SeedTec 317. No flies emerged from any of the 1,060 heads of safflower that we harvested from these three sites, and our dissections of 919 of these heads confirmed that none had been damaged by *Chaetorellia succinea.*

In 1997, we monitored five safflower fields, and a half dozen *Chaetorellia succinea* emerged from 300 safflower heads collected at our site near Red Bluff in Tehama County. At this site, the safflower was grown as a cover crop for dove hunting, and was not harvested. A few days after the first fly emerged from this first sample, we returned to this site and collected a much larger sample (3,217 safflower heads) from which an additional two dozen flies emerged (Table 3).

During 1998, we greatly expanded our sampling, and monitored not only the Red Bluff site, but also an additional 43 sites. A total of 77 flies emerged from the 1998 collection of 1,522 safflower heads from the Red Bluff site. No *Chaetorellia succinea* emerged from the 1,522 safflower heads we collected in 1999 at Red Bluff, but dissection of 102 heads from this sample found a

Table 2. Damage by larvae infesting five safflower varieties and yellow starthistle controls exposed to *Chaetorellia succinea* adults under choice conditions

Test no.	Test duration (days)	*C. succinea*		Variety	Safflower			Yellow starthistle control			χ^2
		Population	n (♀)		Total heads	% of heads infested by *C. succinea*	Infested heads/♀	Total heads	% of heads infested by *C. succinea*	Infested heads/♀	
CH-3-96	14	RC	8	CalWest 4440	53	0	0	384	39.6	19.0	400.64***
CH-4-96	14	RC	8	SeedTec S541	48	0	0	328	42.4	17.4	402.05***
CH-23-99	21	WC	20	CalWest 88-ol	19	0	0	74	91.9	3.70	343.10***
CH-24-99	21	WC	20	CalWest 1221	27	0	0	66	69.9	2.30	183.40***
CH-25-99	21	WC	20	CalWest 88-ol	24	4.2	0.05	87	64.4	2.80	134.05***
CH-34-99	21	WC	13	CalWest 4440	15	0	0	33	9.1	0.23	9.61***
CH-8-00	14	WC	10	Cargill 44	10	0	0	36	41.7	1.50	59.95***
CH-9-00	14	I	10	Cargill 44	6	0	0	30	13.3	0.40	7.70***
CH-13-00	14	SB	10	Cargill 44	6	17	0.10	7	85.7	0.60	59.23***

Chaetorellia succinea populations: (all reared from yellow starthistle, except RC, flies swept from yellow starthistle) RC = Rancho Cordova, Sacramento County, CA. WC = Wildcat Canyon Park, Contra Costa County, CA. I = Ione, Amador County, CA. SB = Sutter's Butte, Butte County, CA. *** $P < 0.001$; chi-squared test of infested versus noninfested heads per female, df = 1.

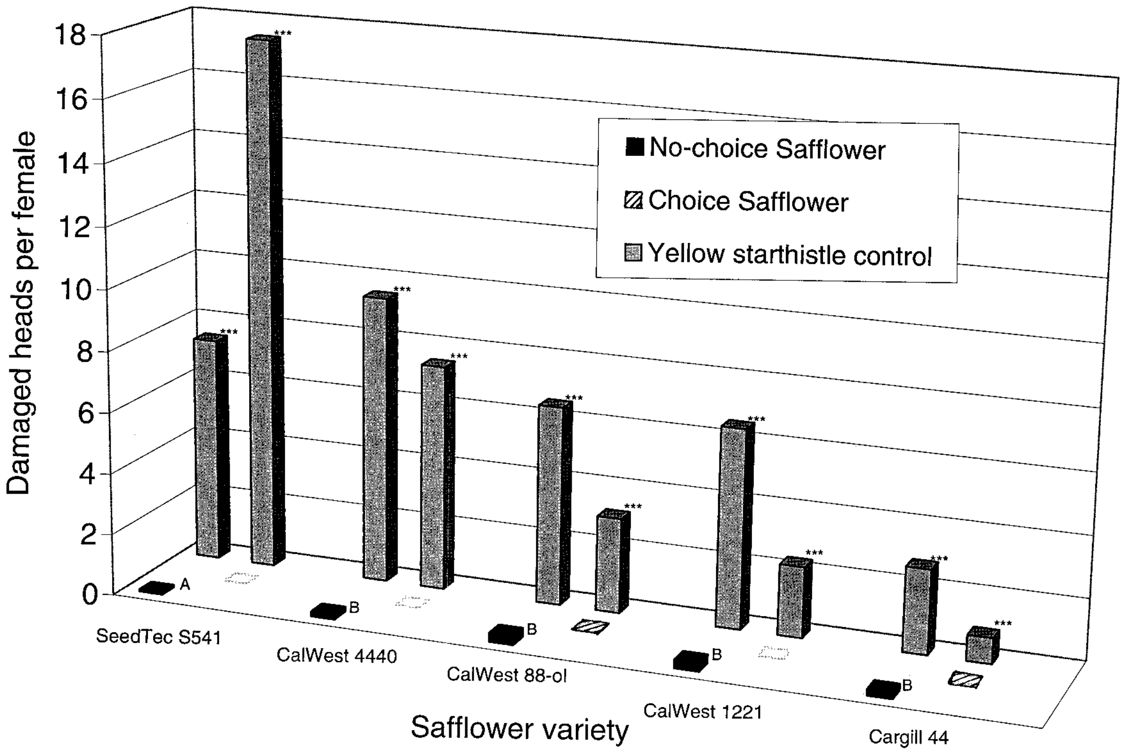

Fig. 1. Amount of larval damage to five varieties of safflower and their paired yellow starthistle controls that were exposed to *Chaetorellia succinea* flies. The height of the bars represents the pooled damage per female. The front row is the pooled no-choice safflower results, and the middle row is the pooled choice safflower results. In the front row, the bars for the damage per female to safflower varieties labeled with the same letter are not statistically different (chi-square comparison of pairs). The back row presents the yellow starthistle controls, with left bar of each variety pair being the pooled damage to yellow starthistle per female during the no-choice tests for that safflower variety, while the right bar depicts the pooled choice test result. Larval damage to yellow starthistle bars that are marked *** were very highly significantly different ($P < 0.001$) from the pooled values for the damage to safflower (the bars in front of each yellow starthistle bar) under no-choice, or choice conditions.

single head, containing three empty *Chaetorellia succinea* pupal cases.

During 1998, a single fly also emerged from nearly 2,500 safflower heads that we collected, in two samples, at a site near Werner in Contra Costa County (Table 3). We dissected all 283 safflower heads from the first sample at this Werner site, and could not find any indication of damage by *Chaetorellia succinea.* Dissections of an additional 520 safflower heads from a later sample from Werner confirmed the lack of *Chaetorellia succinea* there. We now believe that the single fly in our initial sample was collected as an adult, and probably flew there from some distant yellow starthistle site.

No flies emerged from the 14,060 safflower heads we collected at the remaining 45 sites in 1997 and 1998. As it requires approximately three to four minutes to dissect each head and record the results, we lacked the resources to dissect all of the field collected heads to verify the absence of larval damage, dead or overwintering larvae, or empty pupal cases from flies that had emerged before our collection fo the safflower heads.

In dissecting 342 safflower heads from our second 1997 sample at Red Bluff, we found *Chaetorellia* damage in 19 small heads (out of 73), six medium heads (out of 140), and two (out of 110) large heads. chi-square comparison of damage to all head size pairs indicated a very highly significant preference for small heads when compared with medium heads (χ^2 = 19.84, df = 1, $P < 0.001$) or to large heads (χ^2 = 23.0, df = 1, $P < 0.001$). However, because on a safflower plant most of the small heads are concentrated below the top of the plant, we were not certain if perhaps *Chaetorellia succinea* prefers the lower heads.

Therefore, in 1998, while collecting the safflower at our Red Bluff site, we separated the safflower buds by height above the ground, then, within each of the five height classes, by bud size (see *Materials and Methods* section). Our analysis of this 'stratified' sample indicated (Table 4) that although the uppermost layer had the most heads and produced the most flies, there was no statistical difference between height classes. However, during 1998, the number of flies that emerged from both the medium and large safflower heads was statistically significantly higher than from the small heads (small versus medium [χ^2 = 24.6, df = 1, $P <$ 0.001], small versus large [χ^2 = 4.21, df = 1, P = 0.04]). This 'preference reversal' may be due to the unusual

Table 3. Number of *Chaetorellia succinea* flies emerging from safflower heads collected from 47 safflower fields in California, monitored during 1997–1999

County	Site	Date	Plants	Heads	*Ch. succinea* emergence
Butte	Honcut	23 July 1997	—	300	0
Butte	Chico	4 Aug. 1998	30	300	0
Colusa	Grimes	4 Aug. 1998	50	287	0
Colusa	Princeton	4 Aug. 1998	15	316	0
Colusa	Sycamore	4 Aug. 1998	29	302	0
Contra Costa	Brentwood	13 Aug. 1998	50	306	0
Contra Costa	Discovery Bay	13 Aug. 1998	10	285	0
Contra Costa	Werner	13 Aug. 1998	10	283	1
		26 Aug. 1998	250	2,191	0
Fresno	Firebaugh	31 July 1998	18	276	0
		31 July 1998	11	633	0
Glenn	Willows	31 July 1997	—	300	0
Glenn	Afton #1	4 Aug. 1998	26	309	0
Glenn	Afton #2	4 Aug. 1998	24	339	0
Kern	Corcoran #1	30 July 1998	25	338	0
Kern	Delano	30 July 1998	22	369	0
Kern	Wasco	30 July 1998	10	376	0
Kings	Armona	30 July 1998	11	339	0
Kings	Corcoran #2	30 July 1998	35	378	0
Kings	Leemore	30 July 1998	17	360	0
Merced	Gustine	13 Aug. 1998	50	254	0
Merced	Gustine #1[a]	13 Aug. 1998	110	290	0
Merced	Gustine #2[a]	13 Aug. 1998	50	262	0
Monterey	Priest Valley	14 Aug. 1998	10	300	0
Napa	Yountville[a]	21 July 1997	—	300	0
Sacramento	Elverta	6 Aug. 1998	26	293	0
Sacramento	Rio Linda #1	7 Aug. 1998	28	297	0
Sacramento	Rio Linda #2	7 Aug. 1998	17	293	0
San Joaquin	Stockton #1	11 Aug. 1998	17	297	0
San Joaquin	Stockton #2	11 Aug. 1998	48	335	0
San Joaquin	Stockton #3	11 Aug. 1998	24	303	0
San Luis Obispo	Paso Robles #1	13 Aug. 1998	10	84	0
San Luis Obispo	Paso Robles #2	13 Aug. 1998	10	33	0
Santa Clara	San Jose #1	13 Aug. 1998	30	324	0
Santa Clara	San Jose #2	13 Aug. 1998	10	343	0
Shasta	Cottonwood	31 July 1997	—	300	0
		18 Aug. 1998	25	400	0
Solano	Davis	31 July 1998	25	292	0
Solano	Vacaville #1	4 Aug. 1998	28	298	0
Solano	Vacaville #2	7 Aug. 1998	19	300	0
Sutter	Kirkville	6 Aug. 1998	10	362	0
Sutter	Tudor #1	6 Aug. 1998	17	304	0
Sutter	Tudor #2	6 Aug. 1998	16	295	0
Tehama	Red Bluff[a]	31 July 1997	—	300	6
		19 Aug. 1997	—	3,217	24
		7 Aug. 1998	232	1,522	77
		19 July 1999	28	443	1[b]
Tulare	Angiola	30 July 1998	40	270	0
Yolo	Knights Landing	4 Aug. 1998	23	331	0
Yolo	Woodland #1	17 July 1998	59	338	0
Yolo	Woodland #2	31 July 1998	38	290	0
Yuba	Arboga #1	12 Aug. 1998	11	304	0
Yuba	Arboga #2	12 Aug. 1998	10	298	0
		Totals	1,636	22,016	109

—, During 1997, the number of safflower plants was not recorded.
[a] Noncommerical safflower field (see *Materials and Methods*), all other fields were harvested to extract oil from seeds.
[b] 102 safflower heads were dissected; three empty pupal cases were found in one head.

appearance of the safflower at the Red Bluff site in 1998. The grower had relocated the safflower field ≈0.5 km from the field used in 1997. This, along with an unusually cool spring, may have been the reason why the safflower heads were shriveled and contained few seeds (see below).

To assess the damage that *Chaetorellia succinea* was causing to safflower at the Red Bluff site, we dissected a portion of the heads we had collected there each year between 1997 and 1999. During 1997, the 12 *Chaetorellia* damaged heads had a mean of 8.83 seeds, as compared with the mean 19.22 seeds in the 133 undamaged heads. Comparing these two means using an Unpaired *t*-test indicated that they were very highly significantly different ($t = 3.44$, df $= 143$, $P < 0.001$). However, in 1998 the safflower at Red Bluff produced very few seeds—a mean of 1.60 in the 373 undamaged heads, versus 0.67 seeds in the 30 damaged

Table 4. *Chaetorellia succinea* emergence from safflower heads [Cargill-44] collected on 7 August 1998 from Red Bluff, CA

Height class[a]		Head size[b]			Totals
		Small	Medium	Large	
Uppermost 10 cm of 4th quarter	Safflower heads (n)	242	654	92	988
	C. succinea %	1.24%a	7.49%b	3.26%ab	5.67%
Remainder of 4th quarter	Safflower heads (n)	132	243	11	386
	C. succinea %	0%a	6.58%b	0%ab	4.15%
3rd quarter	Safflower heads (n)	55	78	3	136
	C. succinea %	0%a	6.41%ab	33.33%b	3.68%
2nd quarter	Safflower heads (n)	9	3	0	12
	C. succinea %	0%	0%	—	0%
Bottom quarter	Safflower heads (n)	0	0	0	—
			Heads		1,522
			Ch. succinea		77
			% of heads infested		5.06%

[a] Height class: plant stems cut into four equal quarters, top quarter subdivided into uppermost (10 cm) layer, and remainder. Comparison of the % of *Chaetorellia succinea* per head between each height class showed no significant difference ($P < 0.05$ in all comparisons; Z-test for proportions).

[b] Head size diameter: small < 1 cm, median = 1–2 cm, large >2 cm. Within a height class, *Chaetorellia succinea* emergence % followed by the same letter are not significantly different ($P < 0.05$; Z-test for proportions).

heads. This was not significantly different ($t = 1.08$, df = 401, $P = 0.28$). In 1999, infestation rates were too low—only one head out of the 102 dissected had *Chaetorellia* damage—to allow meaningful comparison.

It is interesting to note that the damaged heads per head dissected, was higher than the flies emerged per head collected, for all three years (8.3% damaged heads versus 0.85% emerged flies in 1997; 7.4% damaged against 5.1% emergence in 1998; and the 1.0% damaged versus zero emergence in 1999). We believe that the emergence data consistently underestimated the *Chaetorellia succinea* population because some of the larvae/pupae died before emerging or had emerged before we collected the safflower heads.

To help put this low level of damage to safflower in perspective, in July of 1999, we collected yellow starthistle growing less than 100 m from the safflower at our Red Bluff site. We dissected 262 heads of yellow starthistle, and found that 111 (42%) had been damaged exclusively by *Chaetorellia succinea.* These damaged yellow starthistle heads, contained fewer seeds (mean = 0.45 seeds per head, ± SE 0.125), 91% less than the mean 5.20 seeds (±1.03) found in 98 heads not damaged by *Chaetorellia succinea* (or other arthropods). Not surprisingly, this difference was highly significant statistically (Mann-Whitney rank sum test, $T = 11{,}674$, $n = 98{,}111$, $P < 0.001$).

At almost all of our sites, some safflower plants had minor damage from a variety of other insects and mites, four of which were encountered significantly more frequently than *Chaetorellia succinea* (Table 5). *Chaetorellia succinea* has become a minor component of the arthropod fauna feeding on safflower at one of our 47 safflower sites in California. Even though we collected the safflower late in the growing season, four other minor arthropod pests were more frequently encountered than *Chaetorellia succinea.* If we had collected in the spring or early summer, a different array of even more serious pests would most likely have been found (Kafka and Kearney 1998).

Discussion

Once we had documented the establishment and rapid spread of *Chaetorellia succinea,* a tephritid fly unintentionally introduced into North America (Balciunas and Villegas 1999), we turned our research efforts to documenting the safety of this newly-arrived natural enemy of yellow starthistle, one of the most widespread and pernicious weeds in western United States. Overseas scientists (Zwölfer 1972, Sobhian and Zwölfer 1985), had earlier rejected this fly as a potential biocontrol agent for yellow starthistle because they felt that it might pose a risk to safflower. We, therefore, immediately began testing, both in the laboratory and the field, the susceptibility of various safflower varieties for oviposition and larval development by *Chaetorellia succinea.* Fig. 1 graphically illustrates that yellow starthistle is greatly preferred, usually by several orders of magnitude, by *Chaetorellia succinea* when compared with any of the five varieties of safflower that we tested in the laboratory. However, because under no-choice conditions, all five varieties proved suitable for oviposition and development of *Chaetorellia succinea,* our laboratory results do con-

Table 5. Frequency of herbivorous arthropods commonly found in safflower heads collected at 47 fields in California (1997–1998)

Name	No. sites collected
Homeosoma electellum (Hulst) (Lepidoptera: Pyralidae)	21a
Tetranychus urticae Koch (Acari: Tetranychidae)	17ab
Lygus ssp. complex (Hemiptera: Miridae)	13b
Lasioderma haemorrhoidale (Illiger) (Coleoptera: Anobiidae)	8c
Ch. succinea (Costa) (Diptera: Tephritidae)	2d

Site frequency followed by the same letter are not significantly different ($P < 0.05$; Kruskal-Wallis one-way ANOVA on ranks ($H = 24.6$, df = 4) and Student-Newman-Keuls method for all pairwise multiple comparisons.

firm that this fly is already genetically and physiologically preadapted to potentially use safflower as a host. No-choice tests are widely used in biological control of weeds, primarily to quickly eliminate plants from consideration as potential hosts, and to provide a basis for 'worst case' scenarios. However, positive results in no-choice tests need to be interpreted carefully, because they can lead to misleading conclusions and eliminate a potentially valuable agent (Zwölfer and Harris 1971, Edwards 1999). Choice tests are usually accepted as being better predictors of the risk to a given potential host (Zwölfer and Harris 1971, Cullen 1990, Edwards 1999). Our choice tests did indicate a much reduced risk to safflower. Only one head each of two varieties of safflower was accepted as a host. Thus, based solely on our laboratory results, our best 'prediction' of the risk of the newly introduced *Chaetorellia succinea* to safflower growers was that this fly could establish itself in a safflower field, but if it did, its population levels should be relatively low.

Because *Chaetorellia succinea* was already widely established in Oregon and California, we had the opportunity to compare our laboratory results to actual damage in the field, and to validate our prediction. Our no-choice tests indicated that most of these varieties were susceptible, but the choice tests predicted that only varieties CalWest 88-ol and Cargill 44 might sustain slight damage. None of five varieties of safflower that we planted as 'trap plants' at three *Chaetorellia* infested sites showed any sign of attack by this fly. We believe that this confirms the widely held belief that choice tests more reliably predict the field host range.

Within the subdiscipline of biological control of weeds, most practitioners feel that laboratory tests can indicate a broader array of hosts than the agent will actually use in the field (Zwölfer and Harris 1971, Schroeder 1983, Wapshere 1989, Cullen 1990). Cullen (1990) notes that laboratory tests help determine the "physiological host range"—the array of plants on which the agent might potentially feed or develop. The range of plants which the agent actually utilizes under field conditions is variously referred to as the "true host range" (Harley and Forno 1992), the "ecological host range" (Delfosse 1993), or the "realized host range"(Balciunas et al. 1996).

Our field tests confirm this greater reliability of field host range assessments. None of the varieties we used as trap plants in the field were accepted as hosts by *Chaetorellia succinea.* In addition, during 3 yr of monitoring at 47 safflower fields in 21 counties, we were able to consistently recover *Chaetorellia succinea* from only one site near Red Bluff, CA. The safflower grown there was not harvested, and was a variety, Cargill 44, that we had not previously encountered, and had not been included in our trap plant studies. We added this variety to our laboratory assessments, and it did prove to be significantly more susceptible than the other three varieties we tested under no-choice conditions.

Although populations of *Chaetorellia succinea* have persisted for three years at our Red Bluff site, the infestation rate, as determined from our dissection of safflower heads, has remained low, and declined from 8.3% in 1997 to 7.4% in 1998, to a scarcely detectable 1% in 1999. By comparison, even in 1999, the infestation rate on yellow starthistle growing at the same site was 42%. During 1997, safflower heads at our Red Bluff site that were infested with *Chaetorellia succinea* showed an average seed reduction of 54%. Given the maximum infestation rate of *Chaetorellia succinea* on safflower that we have yet recorded (8.3%), this would indicate a total loss of 4.4% of the safflower seeds to *Chaetorellia succinea* larvae at that site. We feel that few commercial growers would believe that such a small loss would warrant any preventive action on their part.

In July 1996, after we first discovered *Chaetorellia succinea* on safflower, CA. Department of Food and Agriculture (CDFA) issued a Pest Advisory warning growers of our discovery. We are unaware of any reports from growers about this fly, and the preliminary version of our results has reassured agricultural authorities. CDFA is among several agencies that are now considering seeking official regulatory approval for releasing *Chaetorellia succinea* as a biocontrol agent for yellow starthistle. This unusual, after-the-fact, request for approval would allow moving *Chaetorellia succinea* into uninfested areas, and incorporating this fly into yellow starthistle management practices.

In summary, we believe that, in the short term, *Chaetorellia succinea*'s threat to safflower growers in California will be greater than zero, but still very minor. Of course, changes in safflower varieties, cultural practices, and weather might occasionally lead to higher populations of this fly and more damage to safflower than we have thus encountered. Over longer time frames, this fly, as theorized by Sobhian and Zwölfer (1985), may eventually evolve to become better adapted to safflower and then become a significant pest—but so may a large array of other insect species. Mechanisms of evolution, especially those driving host race formation and sympatric speciation have long been a topic of discussion and investigation by tephritid researchers and students of evolution (see Bush 1968, Craig et al. 1993, Feder et al. 1994). Some believe that the addition of apples as a feeding host by *Rhagoletis pomonella* (Walsh) is an example of a recent sympatric speciation (Bernays and Graham 1988, Feder and Bush 1991). But others (Marohasy 1996), believe this to be another example of host substitution. It is beyond the scope of our current study to project or speculate on the impact this fly may have over much longer time periods, as is usually required for evolutionary changes. However, one of the few, and perhaps only (Marohasy 1996), examples of relatively rapid evolution by an insect to accept a new host involved the complete loss of its ancestral host by a specialized butterfly (Singer et al. 1993). In the case of yellow starthistle, we feel that the complete extinction of this ubiquitous weed on a regional level is highly unlikely in the foreseeable future. Thus, one of the most plausible events that would induce the formation of a new host race of *Chaetorellia succinea* that preferred safflower is unlikely to occur.

Fortunately, although this fly's impact on safflower is negligible, it causes far more damage to yellow starthistle, its primary host. It seems likely that *Chaetorellia succinea* will eventually play a significant role in controlling yellow starthistle at some sites. The most important question to resolve is if it will use other western plants, especially the closely related native *Cirsium* thistles and *Centaurea* knapweeds. Further investigations of this fly's impact on yellow starthistle, by itself and in the presence of other agents, such as *Eustenopus villosus*, are warranted. Little is known about this fly's biology, behavior, and ecology, and these need to be determined as well.

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References Cited

Balciunas, J. 1998. The future of biological control for yellow starthistle, pp. 93–95. *In* M. S. Hoddle (ed.), Proceedings, California Conference on Biological Control: Innovations in Biological Control Research, 10–11 June 1998. University of California, Berkeley, CA.

Balciunas, J. K., D. W. Burrows, and M. F. Purcell. 1996. Comparison of the physiological and realized host-ranges of a biological control agent from Australia for the control of the aquatic weed, *Hydrilla verticillata.* Biol. Control 7: 148–158.

Balciunas, J., and B. Villegas. 1999. Two new seed head flies attack yellow starthistle. Calif. Agric. 53: 8–11.

Bernays, E. A., and M. Graham. 1988. On the evolution of host specificity in phytophagous arthropods. Ecology 69: 886–892.

Bush, G. 1968. Sympatric host race formation and speciation in frugivorous flies of the genus *Rhagoletis* (Diptera, Tephritidae). Evolution 23: 237–251.

(CASS) California Agricultural Statistics Service. 2000. 1999 County Agricultural Commissioners' Data. California Agricultural Statistics Service, Sacramento, CA.

Cordy, D. R. 1978. Centaurea species and equine nigropallidal encephalomalacia, pp. 327–336. *In* R. F. Keller, K. R. Van Kampen, and L. F. James (eds.), Effects of poisonous plants on livestock. Academic, New York.

Craig, T. P., J. K. Itami, W. G. Abrahamson, and J. D. Horner. 1993. Behavioral evidence for host-race formation in *Eurosta solidaginis.* Evolution 47: 1696–1710.

Cullen, J. M. 1990. Current problems in host-specificity screening, pp. 27–36. *In* E. S. Delfosse (ed.), Proceedings, VII International Symposium Biological Control Weeds, 6–11 March 1988, Rome, Italy. Istituto Sper. Patologia Veg. (MAF), Rome, Italy.

Delfosse, E. S. 1993. Science and biological control regulation. IOBC Newsl. 58: 1–3.

Edwards, P. B. 1999. The use of choice tests in host-specificity testing of herbivorous insects, pp. 35–43. *In* T. M. Withers, L. B. Browne, and J. Stanley (eds.), Host specificity testing in Australia: towards improved assays for biological control. Scientific Publishing, Indooroopilly, Queensland, Australia.

Ehler, L. E., and L. A. Andres. 1983. Biological control: Exotic natural enemies to control exotic pests, pp. 395–418. *In* C. L. Wilson, and C. L. Graham (eds.), Exotic plant pests and North American agriculture. Academic, New York.

Feder, J. L., and G. L. Bush. 1991. Genetic variation among apples and hawthorn host races of *Rhagoletis pomonella* across an ecological transition zone in the Mid-Western United States. Entomol. Exp. Appl. 59: 249–265.

Feder, J. L., S. B. Opp, B. Wlazlo, K. Reynolds, W. Go, and S. Spisak. 1994. Host fidelity is an effective premating barrier between sympatric races of the apple maggot fly. Proc. Natl. Acad. Sci. USA 91: 7990–7994.

Fuller, T. C., and E. McClintock. 1986. Poisonous plants of California. University of California Press, Berkeley, CA.

Harley, K.L.S., and I. W. Forno. 1992. Biological control of weeds: A handbook for practitioners and students. Inkata, Melbourne, Australia.

Kafka, S. R., and T. E. Kearney. 1998. Safflower production in California. Publication 21565. U.C. Department of Agriculture and Natural Resources, Oakland, CA.

Lamp, C., and F. Collet. 1979. Weeds in Australia. Inkata, Melbourne, Australia.

Maddox, D. M. 1981. Introduction, phenology, and density of yellow starthistle in coastal, intercoastal, and Central Valley situations in California. Agricultural Research Results, ARR-W-20. USDA-ARS, Oakland, CA.

Marohasy, J. 1996. Host shifts in biological weed control: real problems, semantic differences or poor science? Int. J. Pest Manage. 42: 71–75.

Pitcairn, M. J., D. B. Joley, and D. M. Woods. 1998a. Impact of introduced insects for biological control of yellow starthistle, pp. 88–92. *In* M. S. Hoddle (ed.), Proceedings, California Conference on Biological Control: Innovations in Biological Control Research, 10–11 June 1998. University of California, Berkeley, CA.

Pitcairn, M. J., R. A. O'Connell, and J. M. Gendron. 1998b. Yellow starthistle: Survey of statewide distribution, pp. 64–66. *In* D. M. Woods (ed.), Biological control program 1997 summary. California Department of Food and Agriculture, Sacramento, CA.

Schroeder, D. 1983. Biological control of weeds, pp. 41–78. *In* W. W. Fletcher (ed.), Recent advances in weed research. CAB, Tuscon, AZ.

Singer, M. C., C. D. Thomas, and C. Parmesan. 1993. Rapid human-induced evolution of insect-host associations. Nature (Lond.) 366: 681–683.

Sobhian, R., and H. Zwölfer. 1985. Phytophagous insect species associated with flower heads of yellow starthistle (*Centaurea solstitialis*). Z. Angew. Entomol. 99: 301–321.

SPSS. 1997. SigmaStat statistical software, version 2.0 user's manual. SPSS, Chicago, IL.

Turner, C. E., J. B. Johnson, and J. P. McCaffrey. 1995. Yellow starthistle, pp. 270–275. *In* J. R. Nechols, L. A. Andres, J. W. Beardsley, R. D. Goeden, and C. G. Jackson (eds.), Biological control in the Western United States: Accomplishments and benefits of regional research project W-84, 1964–1989. Department of Natural Resources Publ. 3361. University of California, Berkeley, CA.

(USDA-ARS) U.S. Department of Agriculture, Agricultural Research Service. 1970. Selected weeds of the United

States. Agriculture Handbook No. 366. USDA-ARS, Washington, DC.

Wapshere, A. J. 1989. A testing sequence for reducing rejection of potential biological control agents for weeds. Ann. Appl. Biol. 114: 515–526.

Webb, C. J., W. R. Sykes, and P. J. Garnock-Jones. 1988. Flora of New Zealand, volume IV: naturalised pteridophytes, gymnosperms, dicotyledons. Botany Division, Department of Scientific and Industrial Research, Christchurch, New Zealand.

Wells, M. J., A. A. Balsinhas, H. Joffe, V. M. Engelbrecht, G. Harding, and C. H. Stirton. 1986. A catalogue of problem plants in Southern Africa. Memoirs of the Botanical Survey of South Africa, No. 53. Botanical Research Institute, Pretoria, Republic of South Africa.

White, I. M., and K. Marquardt. 1989. A revision of the genus *Chaetorellia* Hendel (Diptera: Tephritidae) including a new species associated with spotted knapweed, *Centaurea maculosa* Lam. (Asteraceae). Bull. Entomol. Res. 79: 453–487.

Zwölfer, H. 1972. Investigations on *Chaetorellia* sp. associated with *C. solstitialis.* Weed project for the University of California. Report No. 7. Commonwealth Institute of Biological Control, Delémont, Switzerland.

Zwölfer, H., and P. Harris. 1971. Host specificity determination of insects for biological control of weeds. Annu. Rev. Entomol. 16: 159–178.

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